

U.S. Department of Commerce Patent and Trademark Office		Attorney's Docket No.
		4933
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. Application No. (if known, see 37 CFR 1.5)
		09/830838
INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE(S) CLAIMED
PCT/EP99/08211	29 October 1999	30 October 1998
TITLE OF INVENTION NEW PRIMERS AND PROBES FOR THE DETECTION OF HIV		
APPLICANT(S) FOR DO/EO/US HABERHAUSEN, KASPER AND KESSLER		
Applicants herewith submit to the United States Designated/Elected Office (DO/EO/US) the following items and other information		
<ol style="list-style-type: none"> 1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C.371 2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371 3. <input checked="" type="checkbox"/> This express request to begin national examination procedures (35 U.S.C. 371(f) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1). 4. <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date. 5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2)) <ul style="list-style-type: none"> a. <input checked="" type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau). b. <input type="checkbox"/> has been transmitted by the International Bureau c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US) 6. <input checked="" type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)). 7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)) <ul style="list-style-type: none"> a. <input checked="" type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau). b. <input type="checkbox"/> have been transmitted by the International Bureau. c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. d. <input type="checkbox"/> have not been made and will not be made. 8. <input checked="" type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)). 9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). [unexecuted] 10. <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)). 		
Items 11 to 16 below concern other document(s) or information included:		
<ol style="list-style-type: none"> 11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98. 12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. 13. <input checked="" type="checkbox"/> A FIRST preliminary amendment. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment. 14. <input type="checkbox"/> A substitute specification. 15. <input type="checkbox"/> A change of power of attorney and/or address letter. 16. Other items or information: 		

U.S. APPLICATION NO. (if known, see 37 CFR 1.5) 09/830838	INTERNATIONAL APPLICATION NO. PCT/EP99/08211	ATTORNEY'S DOCKET NUMBER 4933
17. [X] The following fees are submitted:		<u>CALCULATIONS/PTO USE ONLY</u>
<p>Basic National Fee (37 CFR 1.492(a)(1)-(5)): Search Report has been prepared by the EPO or JPO..... \$860.00</p> <p>International preliminary examination fee paid to USPTO (37 CFR 1.482) \$690.00</p> <p>No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a))..... \$710.00</p> <p>Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO..... \$1,000.00</p> <p>International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4)..... \$100.00</p>		
ENTER APPROPRIATE BASIC FEE AMOUNT =		\$ 860.00
Surcharge of \$130.00 for furnishing the oath or declaration later than [] 20 [] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).		\$
Claims	Number Filed	Number Extra
Total claims	26 - 20 =	6
Independent claims	5 - 3 =	2
Multiple dependent claim(s) (if applicable)		+\$270.00 \$270.00
TOTAL OF ABOVE CALCULATIONS =		\$ 1,398.00
Reduction by 1/2 for filing small entity, if applicable. Verified Small Entity Statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).		\$
SUBTOTAL =		\$ 1,398.00
Processing fee of \$130.00 for furnishing the English translation later than [] 20 [] 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +		\$
TOTAL NATIONAL FEE =		\$
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +		\$
TOTAL FEES ENCLOSED =		\$ 1,398.00
		Amount to be refunded : \$
		charged \$
a. [] A check in the amount of \$ _____ to cover the above fees is enclosed. b. [X] Please charge my Deposit Account No. <u>500812</u> in the amount of \$ <u>1,398.00</u> to cover the above fees. A duplicate copy of this sheet is enclosed. c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>500812</u> . A duplicate copy of this sheet is enclosed.		
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.		
SEND ALL CORRESPONDENCE TO: Victor K. Lee, Ph.D. Roche Molecular Systems, Inc. Customer No. 22829		
Express Mail No. ET059018514US Date: April 30, 2001		 SIGNATURE VICTOR K. LEE, Ph.D. NAME 35,750 REGISTRATION NUMBER

PTO/PCT Rec'd 31 DEC 2001

Express Mail No.: EL 452 481 710 US

HB

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Haberhausen *et al.*

Serial No.: 09/830,838

Art Unit: To be assigned

Based on International Application No.:
PCT/EP99/08211

International Filing Date: 29 October 1999

Examiner: To be assigned

For: New primers and probes for the
detection of HIV

Attorney Docket No.: 4933

**RESPONSE TO NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT
APPLICATIONS CONTAINING NUCLEOTIDE SEQUENCES AND/OR AMINO ACID
SEQUENCE DISCLOSURES**

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In accordance with 37 C.F.R. § 1.821, Applicants, in connection with the above-identified patent application, submit herewith a Sequence Listing in paper and computer readable form pursuant to 37 C.F.R. §§ 1.821(c) and (e), and a copy of the Notice To Comply With Requirements For Patent Applications Containing Nucleotide Sequences And/Or Amino Acid Sequence Disclosures.

I hereby state that the content of the paper and computer readable copies of the Sequence Listing, submitted in accordance with 37 C.F.R. §§ 1.821(c) and (e), respectively, are the same.

No fees are believed due in connection with this response. However, the Commissioner is authorized to charge all required fees, fees under 37 C.F.R. § 1.17 and all required extension of time fees, or credit any overpayment, to Pennie & Edmonds U.S. Deposit Account No. 16-1150 (order no. 1803-296-999).

Respectfully submitted,

Date: October 31, 2001

Rahul Pathak

42,983

(Reg. No.)

For: Jennifer Gordon (Reg. No. 30,753)
PENNIE & EDMONDS LLP
1155 Avenue of the Americas
New York, N.Y. 10036-2711
(212) 790-9090

PTO/PCT Rec'd 31 DEC 2001

#3

Express Mail No.: EL 452 481 710 US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Haberhausen *et al.*

Serial No.: 09/830,838

Examiner: To be assigned

Based on International Application No.:
PCT/EP99/08211

International Filing Date: 29 October 1999

Group Art Unit: To be assigned

For: NEW PRIMERS AND PROBES FOR THE
DETECTION OF HIV

Attorney Docket No.: 4933

PRELIMINARY AMENDMENT UNDER 37 C.F.R. § 1.115

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In accordance with the Rules of Practice, please enter the following amendments and consider the remarks below prior to the examination of the above-captioned application. Filed herewith are Exhibits A-D.

IN THE SPECIFICATION:

A marked up version of the following amended paragraph is attached as Exhibit A. A clean version of the following amended paragraphs is attached hereto as Exhibit B.

On page 11 please replace the paragraph beginning "SEQ ID NO.8: *pol* gene, position 4131-4207..." with the following paragraph:

SEQ ID NO.8: *pol* gene, position 4131-4207, length 76 nucleotides.

IN THE CLAIMS:

A marked up copy of the following amended claim is attached hereto as Exhibit C. A clean version of all pending claims is attached hereto as Exhibit D.

Please amend Claim 13 to read as follows:

13. (Amended) Oligonucleotide, wherein it comprises 10 to 80 consecutive nucleotides from

- (i) a highly conserved region of the *pol* gene of HIV represented by one of the sequences shown in SEQ ID NO: 4, 5, 9 or 10,
- (ii) a corresponding region of another HI virus isolate,
- (iii) a corresponding region of a consensus sequence derived from several HI virus isolates or sequences which are complementary thereto, provided that it does not comprise the nucleotide sequence CTACTACTCC TTGACTTTGG GGATTG (SEQ ID NO: 26) or its complementary sequence.

REMARKS

1. THE AMENDMENT OF THE SPECIFICATION

The specification has been amended to correct a minor error. As the amendment to the specification is fully supported by the specification as originally filed, it does not constitute new matter. Entry thereof is therefore respectfully requested.

2. THE AMENDMENT OF THE CLAIMS

Claim 13 has been amended to incorporate a SEQ ID NO: in the appropriate place. No new matter has been added by this amendment. Entry thereof is therefore respectfully requested.

A marked up version of the amended claim is attached hereto as Exhibit C. For the PTO's convenience, a clean copy of the pending claims after entry of the instant amendment is attached hereto as Exhibit D.

CONCLUSION

Applicants submit that Claims 1-21 satisfy all of the criteria for patentability and are in condition for allowance. An early indication of the same and passage of Claims 1-21 to issuance is therefore kindly solicited.

No fees are believed due in connection with this response. However, the Commissioner is authorized to charge all required fees, fees under 37 CFR § 1.17 and credit any overpayment, to Pennie & Edmonds U.S. Deposit Account No. 16-1150 (1803-296-999).

Respectfully submitted,

Date: October 31, 2001


Rahul Pathak 42,983
(Reg. No.)

For: Jennifer Gordon (Reg. No.30,753)
PENNIE & EDMONDS LLP
1155 Avenue of the Americas
New York, New York 10036-2711
(212) 790-9090

~~CONFIDENTIAL~~

EXHIBIT A

MARKED UP VERSION OF REPLACEMENT PARAGRAPH

On page 11 please replace the paragraph beginning “SEQ ID NO.8: *pol* gene, position 4131-4207...” with the following paragraph:

SEQ ID NO.8: *pol* gene, position 4131-4207, length [77] 76 nucleotides.

EXHIBIT B

REPLACEMENT PARAGRAPH

On page 11 please replace the paragraph beginning “SEQ ID NO.8: *pol* gene, position 4131-4207...” with the following paragraph:

SEQ ID NO.8: *pol* gene, position 4131-4207, length 76 nucleotides.

EXHIBIT C

MARKED VERSION OF THE AMENDED CLAIM

13. (Amended) Oligonucleotide, wherein it comprises 10 to 80 consecutive nucleotides from

- (i) a highly conserved region of the *pol* gene of HIV represented by one of the sequences shown in SEQ ID NO: 4, 5, 9 or 10,
- (ii) a corresponding region of another HI virus isolate,
- (iii) a corresponding region of a consensus sequence derived from several HI virus isolates or sequences which are complementary thereto, provided that it does not comprise the nucleotide sequence
CTACTACTCC TTGACTTTGG GGATTG (SEQ ID NO: 26)
or its complementary sequence.

EXHIBIT D

PENDING CLAIMS UPON ENTRY OF INSTANT AMENDMENT

1. Method for the subtype-independent and/or species-independent detection of nucleic acids of HI viruses in a sample by hybridizing the nucleic acids with an oligonucleotide combination comprising two or more oligonucleotides which hybridize specifically with HIV nucleic acids and contain in each case 10 to 80 consecutive nucleotides from
 - (i) the same highly conserved region of the LTR region, of the *gag* gene or of the *pol* gene of HIV represented by one of the sequences shown in SEQ ID NO: 1 to 13,
 - (ii) a corresponding region of another HI virus isolate,
 - (iii) a corresponding region of a consensus sequence derived from several HI virus isolates or sequences which are complementary thereto, and carrying out an enzymatic amplification step.
2. Method as claimed in claim 1, wherein it comprises the steps:
 - (a) contacting a sample with the oligonucleotides under such conditions that the oligonucleotides hybridize with the HIV nucleic acids from HIV-1 or/and HIV-2 that are present in the sample,
 - (b) determining the presence and/or the amount of HIV nucleic acids in the sample.

3. Method as claimed in claim 1 or 2,

wherein

only a single oligonucleotide combination is used.

4. Method as claimed in one of the claims 1 to 3,

wherein

the oligonucleotides are selected for a subtype-independent detection in such a manner that at least 7 of the HIV-1 subtypes selected from the subtypes A, B, C, D, E, F, G, H and O and at least 2 of the HIV-2 subtypes selected from the subtypes A, B, C and D are detected.

5. Method as claimed in one of the claims 1 to 3,

wherein

the oligonucleotides are selected for a species-independent detection in such a manner that at least 7 of the HIV-1 subtypes selected from the subtypes A, B, C, D, E, F, G, H and O and additionally at least one of the HIV-2 subtypes selected from the subtypes A, B, C and D are detected.

6. Method for the subtype-independent and/or species-independent detection of nucleic acids of HI viruses in a sample by hybridizing the nucleic acids with two or more oligonucleotide combinations, each oligonucleotide combination comprising a first oligonucleotide which comprises 10 to 80 consecutive nucleotides from

(i) a highly conserved region of the LTR region,

of the *gag* gene or of the *pol* gene of HIV

represented by one of the sequences shown in

SEQ ID NO: 1 to 13,

(ii) a corresponding region of another HI virus isolate ,

(iii) a corresponding region of a consensus sequence derived from several HI virus isolates, or sequences which are complementary thereto, and a second oligonucleotide which enables subtype-specific and/or species-specific hybridization with HIV nucleic acids, and carrying out an enzymatic amplification step, wherein the entirety of the oligonucleotide combinations allows a subtype-independent and/or species-independent detection of HI viruses.

7. Method as claimed in claim 6,

wherein

the oligonucleotides are selected for the subtype-independent detection in such a manner that at least 7 of the HIV-1 subtypes selected from the subtypes A, B, C, D, E, F, G, H and O and at least 2 of the HIV-2 subtypes selected from the subtypes A, B, C and D are detected.

8. Method as claimed in claim 7,

wherein

at least two oligonucleotides are used for the detection which contain in each case 10 to 80 consecutive nucleotides from

(i) a highly conserved region of the LTR gene, of the *gag* gene or of the *pol* gene of HIV

represented by one of the sequences shown in
SEQ ID NO: 2, 4, 5, 6, 8, 9, 10, 12 and 13,
(ii) a corresponding region of another HI virus
isolate ,
(iii) a corresponding region of a consensus sequence
derived from several HI virus isolates
or sequences which are complementary thereto.

9. Method as claimed in claim 6,

wherein

the oligonucleotides are selected for the species-independent detection in such a manner that at least 7 of the HIV-1 subtypes selected from the subtypes A, B, C, D, E, F, G, H and O and additionally at least one of the HIV-2 subtypes selected from the subtypes A, B, C and D are detected.

10. Method as claimed in claim 9,

wherein

at least two oligonucleotides are used for the detection which contain in each case 10 to 80 consecutive nucleotides from

- (i) a highly conserved region of the LTR gene,
of the *gag* gene or of the *pol* gene of HIV
represented by one of the sequences shown in
SEQ ID NO: 1, 2, 3, 4, 5, 7, 9, 10 and 13,
- (ii) a corresponding region of another HI virus

isolate,

(iii) a corresponding region of a consensus sequence derived from several HI virus isolates or sequences which are complementary thereto.

11. Method as claimed in one of the previous claims, wherein the oligonucleotides have or contain the sequences shown in SEQ ID NO. 14 to 25.
12. Method as claimed in one of the previous claims, wherein at least one oligonucleotide has one or several labels.
13. (Amended) Oligonucleotide, wherein it comprises 10 to 80 consecutive nucleotides from
 - (i) a highly conserved region of the *pol* gene of HIV represented by one of the sequences shown in SEQ ID NO: 4, 5, 9 or 10,
 - (ii) a corresponding region of another HI virus isolate,
 - (iii) a corresponding region of a consensus sequence derived from several HI virus isolates or sequences which are complementary thereto, provided that it does not

comprise the nucleotide sequence

CTACTACTCC TTGACTTTGG GGATTG (SEQ ID NO: 26)

or its complementary sequence.

14. Oligonucleotide as claimed in claim 13,

wherein

it comprises 10 to 80 consecutive nucleotides from

(i) a highly conserved region of the *pol* gene of
HIV represented by one of the sequences shown

in SEQ ID NO: 4, 5 or 9,

(ii) a corresponding region of another HI virus

isolate,

(iii) a corresponding region of a consensus

sequence derived from several HI virus

isolates

or sequences which are complementary thereto.

15. Oligonucleotide,

wherein

it comprises at least one of the sequences shown in SEQ ID NO. 14, 16, 17, 18, 20,
22, 23, 24 and 25.

16. Oligonucleotide as claimed in one of the claims 13 to 15,

wherein

it has no mismatches at its 3' end with nucleic acids of the subtypes A, B, C, D, E, F, G, H and O of HIV-1 and of the subtypes A, B, C and D of HIV-2.

17. Oligonucleotide as claimed in one of the claims 13 to 16,

wherein

it has one or several labels.

18. Combination of several oligonucleotides comprising at least two oligonucleotides,

wherein

the at least two oligonucleotides each comprise 10 to 80 consecutive nucleotides from

(i) a highly conserved region of the LTR region,

of the *gag* gene or of the *pol* gene of HIV

represented by one of the sequences shown in

SEQ ID NO: 1 to 13,

(ii) a corresponding region of another HI virus

isolate,

(iii) a corresponding region of a consensus

sequence derived from several HI virus

isolates

or sequences which are complementary thereto and the combination is selected such that it allows an enzymatic amplification.

19. Combination of several oligonucleotides comprising at least two oligonucleotides

selected from the oligonucleotides as claimed in one of the claims 13 to 17 and

optionally additional oligonucleotides which each contain a sequence that is specific

for a single subtype of HIV-1 and/or HIV-2, wherein the entirety of the oligonucleotides allows a subtype-independent and/or species-independent detection of HI viruses.

20. Reagent kit comprising an oligonucleotide as claimed in one of the claims 13 to 17 or an oligonucleotide combination as claimed in claim 18 or 19 as primers and/or probes for the detection of HI viruses or their nucleic acids and suitable means for carrying out a hybridization and amplification of nucleic acids in a sample.
21. Use of oligonucleotides or oligonucleotide combinations as claimed in one of the claims 13 to 19 as primers and/or probes for the subtype-independent and/or species-independent detection of HI viruses.

09/830838
JC08 Rec'd PCT/PTO 30 APR 2001
PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application
based on International Application No. PCT/EP99/08211
Filed October 29, 1999
Inventor(s) HABERHAUSEN *et al.*

For: **NEW PRIMERS AND PROBES FOR THE DETECTION OF HIV**

Attorney Docket No.: 4933

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Box PCT
Washington, D.C. 20231

Alameda, CA 94501
April 30, 2001

Sir:

Prior to examining the above-referenced application as entering the National Stage under 35 U.S.C. §371, please consider the following amendments and remarks.

IN THE CLAIMS:

At page 32, line 1, of the English translation of the International Application, please delete "Claims" and insert therefor --WHAT IS CLAIMED IS:--.

Please amend the claims as follows:

1. (Amended) A method [Method] for the subtype-independent [and/]or species-independent detection of nucleic acids of HI viruses in a sample comprising [by]
(a) hybridizing the nucleic acids with an oligonucleotide combination comprising two or more oligonucleotides which hybridize specifically with HIV nucleic

acids and contain in each case 10 to 80 consecutive nucleotides from

- (i) the same highly conserved region of the LTR region, of the *gag* gene or of the *pol* gene of HIV represented by one of the sequences shown in SEQ ID NO: 1 to 13,
- (ii) a corresponding region of another HI virus isolate,
- (iii) a corresponding region of a consensus sequence derived from several HI virus isolates or sequences which are complementary thereto, and

- (b) carrying out an enzymatic amplification step.

2. (Amended) The method [Method] as claimed in claim 1, further comprising [wherein it comprises] the steps:

- (a) contacting the [a] sample with the oligonucleotides under such conditions that the oligonucleotides hybridize with the HIV nucleic acids from HIV-1 or/and HIV-2 that are present in the sample, and
- (b) determining the presence and/or the amount of HIV nucleic acids in the sample.

3. (Amended) The method [Method] as claimed in claim 1 [or 2], wherein only a single oligonucleotide combination is used.

4. (Amended) The method [Method] as claimed in claim 1 [one of the claims 1 to 3], wherein the oligonucleotides are selected for a subtype-independent detection in such a manner that at least 7 of the HIV-1 subtypes selected from the subtypes A, B, C, D, E, F, G, H and O and at least 2 of the HIV-2 subtypes selected from the subtypes A, B, C and D are detected.

5. (Amended) The method [Method] as claimed in claim 1 [one of the claims 1 to 3], wherein the oligonucleotides are selected for a species-independent detection in such a manner that at least 7 of the HIV-1 subtypes selected from the subtypes A, B, C, D, E, F, G, H and O and additionally at least one of the HIV-2 subtypes selected from the subtypes A, B, C and D are detected.

6. (Amended) A method [Method] for the subtype-independent [and/]or species-independent detection of nucleic acids of HI viruses in a sample comprising [by]

- (a) hybridizing the nucleic acids with two or more oligonucleotide combinations, each oligonucleotide combination comprising a first oligonucleotide which comprises

10 to 80 consecutive nucleotides from

- (i) a highly conserved region of the LTR region, of the *gag* gene or of the *pol* gene of HIV represented by one of the sequences shown in SEQ ID NO: 1 to 13,
- (ii) a corresponding region of another HI virus isolate,
- (iii) a corresponding region of a consensus sequence derived from several HI virus isolates, or sequences which are complementary thereto, and a second oligonucleotide which enables subtype-specific [and/]or species-specific hybridization with HIV nucleic acids, and

(b) carrying out an enzymatic amplification step, wherein the entirety of the oligonucleotide combinations allows a subtype-independent [and/]or species-independent detection of HI viruses.

7. (Amended) The method [Method] as claimed in claim 6, wherein the oligonucleotides are selected for the subtype-independent detection in such a manner that at least 7 of the HIV-1 subtypes selected from the subtypes A, B, C, D, E, F, G, H and O and at least 2 of the HIV-2 subtypes selected from the subtypes A, B, C and D are detected.

8. (Amended) The method [Method] as claimed in claim 7, wherein at least two oligonucleotides are used for the detection which contain in each case 10 to 80 consecutive nucleotides from

- (i) a highly conserved region of the LTR gene, of the *gag* gene or of the *pol* gene of HIV represented by one of the sequences shown in SEQ ID NO: 2, 4, 5, 6, 8, 9, 10, 12 and 13,
- (ii) a corresponding region of another HI virus isolate,
- (iii) a corresponding region of a consensus sequence derived from several HI virus isolates or sequences which are complementary thereto.

9. (Amended) The method [Method] as claimed in claim 6, wherein the oligonucleotides are selected for the species-independent detection in such a manner that at least 7 of the HIV-1 subtypes selected from the subtypes A, B, C, D, E, F, G, H and O and additionally at least one of the HIV-2 subtypes selected from the subtypes A, B, C and D are detected.

10. (Amended) The method [Method] as claimed in claim 9, wherein at least two oligonucleotides are used for the detection which contain in each case 10 to 80 consecutive nucleotides from

- (i) a highly conserved region of the LTR gene, of the *gag* gene or of the *pol* gene of HIV represented by one of the sequences shown in SEQ ID NO: 1, 2, 3, 4, 5, 7, 9, 10 and 13,
- (ii) a corresponding region of another HI virus isolate,
- (iii) a corresponding region of a consensus sequence derived from several HI virus isolates or sequences which are complementary thereto.

11. (Amended) The method of claim 1, [Method as claimed in one of the previous claims,] wherein the oligonucleotides have or contain the sequences shown in SEQ ID NO. 14 to 25.

12. (Amended) The method of claim 1, [Method as claimed in one of the previous claims,] wherein at least one oligonucleotide has one or several labels.

13. (Amended) An oligonucleotide comprising [Oligonucleotide, wherein it comprises] 10 to 80 consecutive nucleotides from

- (i) a highly conserved region of the *pol* gene of HIV represented by one of the sequences shown in SEQ ID NO: 4, 5, 9 or 10,
- (ii) a corresponding region of another HI virus isolate,
- (iii) a corresponding region of a consensus sequence derived from several HI virus isolates or sequences which are complementary thereto, provided that it does not comprise the nucleotide sequence
CTACTACTCC TTGACTTTGG GGATTG
or its complementary sequence.

14. (Amended) The oligonucleotide [Oligonucleotide] as claimed in claim 13, [wherein it comprises] comprising 10 to 80 consecutive nucleotides from

- (i) a highly conserved region of the *pol* gene of HIV represented by one of the sequences shown in SEQ ID NO: 4, 5 or 9,
- (ii) a corresponding region of another HI virus isolate,
- (iii) a corresponding region of a consensus sequence derived from several HI virus isolates or sequences which are complementary thereto.

15. (Amended) An oligonucleotide comprising [Oligonucleotide, wherein it comprises at least] one of the sequences shown in SEQ ID NO. 14, 16, 17, 18, 20, 22, 23, 24 and 25.

16. (Amended) The oligonucleotide of claim 13 or 15 [Oligonucleotide as claimed in one of the claims 13 to 15,] wherein it has no mismatches at its 3' end with nucleic acids of the subtypes A, B, C, D, E, F, G, H and O of HIV-1 and of the subtypes A, B, C and D of HIV-2.

17. (Amended) The oligonucleotide of claim 13 or 15 [Oligonucleotide as claimed in one of the claims 13 to 16,] wherein it has one or several labels.

18. (Amended) An oligonucleotide combination [Combination of several oligonucleotides] comprising at least two oligonucleotides, wherein [the at least two oligonucleotides] each oligonucleotide comprises [comprise] 10 to 80 consecutive nucleotides from

- (i) a highly conserved region of the LTR region, of the *gag* gene or of the *pol* gene of HIV represented by one of the sequences shown in SEQ ID NO: 1 to 13,
- (ii) a corresponding region of another HI virus isolate,
- (iii) a corresponding region of a consensus sequence derived from several HI virus isolates or sequences which are complementary thereto and the combination is selected such that it allows an enzymatic amplification.

19. (Amended) An oligonucleotide combination [Combination of several oligonucleotides] comprising at least two oligonucleotides selected from the oligonucleotides as claimed in claim 13 or 15 [one of the claims 13 to 17] and optionally additional oligonucleotides each of which contains [which each contain] a sequence that is specific for a single subtype of HIV-1 and/or HIV-2, wherein the entirety of the oligonucleotides allows a subtype-independent and/or species-independent detection of HI viruses.

20. (Amended) A [Reagent] kit comprising an oligonucleotide as claimed in claim 13 or 15 [one of the claims 13 to 17 or an oligonucleotide combination as claimed in claim 18 or 19] as primers and/or probes for the detection of HI viruses or their nucleic

acids and suitable means for carrying out a hybridization and amplification of nucleic acids in a sample.

21. (Amended) A method of using [Use of] oligonucleotides [or oligonucleotide combinations] as claimed in claim 13 or 15 [one of the claims 13 to 19] as primers and/or probes for the subtype-independent and/or species-independent detection of HI viruses.

REMARKS

Applicants have amended the claims to comply with U.S. patent practice in matters of form and to remove certain multiple dependency. After entry of this Amendment, claims 1-21 are pending in this application. The amendments do not introduce new matter. Entry of this Amendment is respectfully requested.

The total filing fee on the Transmittal Letter To The United States Designated/Elected Office (DO/EO/US) Concerning A Filing Under 35 U.S.C. §371 is calculated on the basis of this Amendment.

Respectfully submitted

By: 

Victor K. Lee, (Reg. No. 35,750)
Attorney for Applicants
Roche Molecular Systems, Inc.
Customer No. 22829
Telephone (510) 814-2966
Telefax (510) 814-2973

18657P WO
Roche Diagnostics GmbH
PCT/EP99/08211

JC08 Rec'd PCT/PTO 30 APR 2007

New Claims 13 and 14

13. Oligonucleotide,

wherein

it comprises 10 to 80 consecutive nucleotides from

- (i) a highly conserved region of the *pol* gene of HIV represented by one of the sequences shown in SEQ ID NO: 4, 5, 9 or 10,
- (ii) a corresponding region of another HI virus isolate,
- (iii) a corresponding region of a consensus sequence derived from several HI virus isolates

or sequences which are complementary thereto, provided that it does not comprise the nucleotide sequence

CTACTACTCC TTGACTTTGG GGATTG

or its complementary sequence.

14. Oligonucleotide as claimed in claim 13,

wherein

it comprises 10 to 80 consecutive nucleotides from

- (i) a highly conserved region of the *pol* gene of HIV represented by one of the sequences shown in SEQ ID NO: 4, 5 or 9,
- (ii) a corresponding region of another HI virus isolate,
- (iii) a corresponding region of a consensus sequence derived from several HI virus isolates

or sequences which are complementary thereto.

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New primers and probes for the detection of HIV

Description

The invention concerns a method for the subtype-independent or/and species-independent detection of HIV viruses in a sample and suitable oligonucleotides for this.

The detection of HIV is of major importance in analytical diagnostics. There are a number of detection methods which are based on the immunological detection of HIV-specific antibodies, HIV proteins such as reverse transcriptase or HIV-specific nucleic acids.

Since HIV and its genetic material only occurs in very low concentrations in body fluids, the sensitivity of the detection method is an important factor for the usability of such methods.

For this reason PCR (polymerase chain reaction) which is based on an amplification of the nucleic acids to be detected is now being used to an increasing extent. Applications of this method for the direct detection of HIV are described for example in EP-B-0 200 362 and EP-B-0 201 184.

PCR or the polymerase chain reaction allows the amplification of nucleic acid sections with the aid of oligonucleotides, so-called primers, which hybridize specifically with the nucleic acids to be detected. Amplification products are formed in this process which in turn can be detected with other specific

oligonucleotides, so-called probes.

Prerequisites for a successful PCR for the detection of HIV are on the one hand that the complementary base sequence of the primers used agree as exactly as possible with that of the nucleic acid to be detected so that the hybridization is as specific as possible. However, on the other hand it is also advantageous to be able to amplify as many variants of HIV as possible with the same primers.

Since the discovery of HIV-1 it has been found that there are differences in the nucleic acid sequences of HIV viruses of different origins. The various types of HIV-1 are usually referred to as subtypes. At present at least 9 subtypes are known which are referred to as subtypes A to H and O (Human Retroviruses and AIDS, Los Alamos, Natl. Laboratory, Los Alamos, New Mexico, 1994; Publisher G. Meyers et al., I-A-1). No primers or probes have yet been developed that can recognize all known subtypes of HIV-1. In addition it would also be advantageous to be able to also detect HIV-2 and its subtypes with the same primers and probes. At present the HIV-2 subtypes A, B, C and D are known.

In some patent applications oligonucleotide primers and/or probes have been disclosed which, however, are not encompassed by the present application.

WO96/02557 describes general uses of oligonucleotides to inhibit HIV propagation and to detect the virus. However, there is no reference to a suitability for a species-independent or subtype-independent detection.

Primers and probes are described in the European Patent Application EP 0 403 333 which each hybridize with base sequences from conserved regions of the *gag*, *vpr* and *pol* genes of the HIV-1 isolates Bru, Mal and Eli and with corresponding regions of HIV-2 ROD and SIV Mac. In addition primers and probes are disclosed which each hybridize with sections from the *env*, *nef1*, *vif1* and *vpr* genes of HIV-1 Bru, Mal and Eli and those which hybridize with sections from the *nef2*, *vif2* and *vpx* genes of HIV-2 ROD and SIV Mac. Although some of these oligonucleotides apparently hybridize independent of species, no statements are made about which subtypes of the individual viruses are recognized.

Further detection methods for HIV are disclosed for example in EP 839 917 which only enables subtypes of HIV-1 to be detected as well as in the applications EP 887 427, WO99/07898 and WO98/58086 which were published later. Primers and probes are disclosed in the European Patent Application EP 727 497 which amplify a sequence section for the *pol* gene of HIV-1 and can thus recognize five subtypes of HIV-1.

EP 0 617 132 also disclose primers and probes for the detection of HIV-1 which are able to distinguish between HIV-1 and its phylogenetically close relatives such as HTLV-II or HIV-2. The oligonucleotides selected in this case hybridize with a number of regions from the HIV genome including LTR and most of the structural genes. However, there are no references to a suitability for a subtype-independent detection.

In addition there are methods in the prior art which use oligonucleotides for a purification by means of

hybridization with target oligos e.g. WO98/27425. Such oligos are, however, not suitable for a subtype-independent detection of HIV.

Although WO90/01069 discloses oligonucleotide pairs for the detection of HIV, these pairs are not used as primers for an enzymatic amplification but rather hybridize with the same strand and themselves represent the sought-after sequence after their linkage.

Since previously there has not yet been a single primer pair that is suitable for a subtype-independent or/and species-independent detection, several primer pairs are now frequently used for the amplification. However, this results, on the one hand, in increased reagent costs and, on the other hand, in a much more complex PCR.

Hence the object of the present invention was to provide a method for the subtype-independent or/and species-independent detection of HI viruses in a sample. In particular it was an object of the invention to provide a method that enables more subtypes of HIV-1 and/or HIV-2 to be detected than was previously possible.

This object is achieved according to the invention by a method for the subtype-independent and/or species-independent detection of nucleic acids of HI viruses in a sample by

hybridizing the nucleic acids with at least one oligonucleotide hybridizing specifically with HIV nucleic acids which contains at least 10 consecutive nucleotides from

- (i) a highly conserved region of the LTR region, of the gag gene or of the pol gene of HIV

represented by one of the sequences shown in SEQ ID NO: 1 to 13,

- (ii) a corresponding region of another HI virus isolate,
- (iii) a corresponding region of a consensus sequence from several HI virus isolates, or sequences which are complementary to these.

This object is preferably achieved by a method for the subtype-independent and/or species-independent detection of nucleic acids of HI viruses in a sample by hybridizing the nucleic acids with an oligonucleotide combination comprising two or several oligonucleotides hybridizing specifically with HIV nucleic acids which each contain at least 10 consecutive nucleotides from

- (i) a highly conserved region of the LTR region, of the *gag* gene or of the *pol* gene of HIV represented by one of the sequences shown in SEQ ID NO: 1 to 13,
- (ii) a corresponding region of another HI virus isolate,
- (iii) a corresponding region from a consensus sequence derived from several HI virus isolates,

or sequences that are complementary to these, and performing an amplification step.

The method according to the invention enables more subtypes of HIV-1 and HIV-2 (subtype-independent detection) to be detected than was previously possible with the prior art. Subtype-independent detection means that at least 2 subtypes of a respective species can be detected with a single probe or with a single oligonucleotide combination. As already mentioned above

it has previously not been possible to detect subtypes A, B, C, D, E, F, G, H and O of HIV-1 with a single probe or with a single amplification primer pair composed of two oligonucleotides using conventional detection methods. The method according to the invention now enables at least 7 of 9 subtypes of HIV-1, in particular including subtype O together with other subtypes, to be detected with the aid of very few oligonucleotides and in the best case with the same oligonucleotides. In a preferred embodiment it is possible to detect all previously known 9 subtypes. The subtype-independent test enables the detection of at least 2, preferably at least 3 and most preferably all subtypes A, B, C and D of HIV-2. In addition to the subtype-independent detection it is also possible to detect nucleic acids of HIV-1 and HIV-2 independent of species. Species-independent means that various species of immunodeficient viruses can be detected with the same oligonucleotides e.g. HIV-1 and HIV-2. Preferably at least 7 of the presently known 9 subtypes of HIV-1 and other presently known subtypes of HIV-2 are detected. Particularly preferably it is possible to detect all 9 subtypes of HIV-1 plus further subtypes of HIV-2 and particularly preferably plus all currently known subtypes of HIV-2.

The method according to the invention is based on an amplification of nucleic acid sections of HI viruses with the aid of specific oligonucleotides which can act as primers or as probes.

An oligonucleotide is a single-stranded linear nucleic acid molecule. In general oligonucleotides have 10 to 100 bases. The oligonucleotides according to the invention are preferably 10 to 80, particularly

preferably 10 to 60 and more preferably 10 to 30 and most preferably 20 to 30 nucleotides long. As with nucleic acids one distinguishes here between oligodeoxyribonucleotides and oligoribonucleotides; however, oligoribonucleotides also include compounds in which the hydrogen of the hydroxy group is replaced by organic residues such as an allyl group. Such compounds have been known for a long time to a person skilled in the art. In addition the term "oligonucleotide" can also refer to molecules in which the sugar phosphate backbone is replaced by a peptide backbone. This group of compounds is referred to as PNA. A common feature of all oligonucleotides is that they have bases on the backbone which are able to form hydrogen bonds with complementary bases. The bases include the natural bases A, G, C, T and U and also artificial bases such as deaza-G.

An oligonucleotide primer is an oligonucleotide which can hybridize with a second likewise single-stranded nucleic acid and subsequently be extended with nucleoside triphosphates by a suitable enzyme e.g. a DNA polymerase along the second nucleic acid which serves as a template such that a double-stranded nucleic acid is formed. Hence the primers normally have a hydroxyl group on the 3' C atom of the sugar at the 3' end i.e. at the end where the nucleotide building blocks are attached.

An oligonucleotide combination comprises several oligonucleotides and it preferably comprises a primer pair or a combination of primers and probes such as a primer pair and a probe. A primer pair is composed of two oligonucleotide primers which allow the, preferably enzymatic, amplification of a certain section of a nucleic acid. Preferably both primers of the primer pair hybridize with different strands of the original nucleic

acid such that the respective extension products overlap each other. As a result each primer can hybridize with the extension product of the other primer and a section between the two primers is multiplied as an amplification product. So-called probes are also single-stranded oligonucleotides which, although also acting as primers, are mainly used to specifically hybridize with already amplified nucleic acid sections in order to thus enable a nucleic acid detection. The name oligonucleotide thus encompasses the term primer and probe which only differ in their function. The oligonucleotides used in the method according to the invention can also contain marker groups such as radioactive labels or fluorescent labels. It is especially the probes which have labels.

Amplification is understood as a multiplication of nucleic acids or sections of nucleic acids. A known amplification method is the already mentioned PCR. In this method a normally double-stranded DNA molecule is firstly denatured i.e. is split into its single strands. Then the amplification primers are added under conditions which allow hybridization of the primers with the target DNA sequence. The primers are then extended along the nucleic acid template with the aid of a polymerase enzyme and nucleotide building blocks. Subsequently the newly formed double-stranded nucleic acids are again denatured and a new polymerase cycle begins. Conventional PCR methods typically use between 25 and 40 cycles. An amplification in the sense of the invention can also include necessary preparation steps for the nucleic acid amplification such as the denaturation of the double-stranded DNA.

In this application the term "hybridization" denotes the

joining of two complementary nucleic acid single strands to form a double strand. The single strands do not have to be 100 % complementary for this but can have deviations in their base sequence. In order to achieve a suitable hybridization for the method according to the invention the single strands, in this case the oligonucleotides, must, however, be sufficiently specific under the prevailing hybridization conditions.

In order to ensure the advantages mentioned above of the method according to the invention described here, the oligonucleotides that are used must fulfil at least two conditions. On the one hand they must be sufficiently specific to exclusively hybridize with nucleic acids which are derived from HI viruses. On the other hand these viruses vary very greatly in some genome regions i.e. there are relatively large differences in the base sequence. On the basis of these differences one distinguishes on the one hand between HIV-1 and HIV-2 as well as between so-called subtypes which represent relatively closely related strains of the same virus. With reference to the oligonucleotides of the present invention this means that these must also be able to not only recognize a particular virus or a particular subtype but they must also have a base sequence that allows hybridization with as many subtypes of HIV-1 or HIV-2 as possible or even with all known subtypes or even hybridization with both.

For this purpose one usually selects oligonucleotides from relatively conserved regions of the genome of the viruses to be detected and then uses the respective complementary sequence. Some highly conserved regions are already known from the prior art (see above). Conserved regions are sections of nucleic acid on the

genome of HIV which, compared to the remainder of the genome, only have very slight differences in the base sequence. This is also referred to as base identity which is expressed in percent or as homology.

Surprisingly oligonucleotides were found in the present invention which allow a subtype-independent detection as well as a species-independent detection of HIV. These oligonucleotides hybridize with newly discovered highly conserved regions of HIV. These are located in the LTR region, the *gag* gene and the *pol* gene. These regions are relatively small but, having an average length of 50 to 100 nucleotides, they allow hybridization with one or several oligonucleotides. As in most of the cited documents of the prior art the location of the regions of the HIV genome in this application is based on the numbering of the HIV-1 isolate HXB2 as published in : Wong-Staal et al., *Nature* 313, 277-284 (1985). The sequences of these new highly conserved regions are in each case shown in SEQ ID NO. 1 to 13, the sequences of which refer to the sequence of HIV-1 HXB2, accession number K03455 of the HIV sequence data base (<http://HIV-web.lanl.gov/>). The position of the highly conserved sequences is as follows:

SEQ ID NO.1: LTR region, position 504-565, length 62 nucleotides,

SEQ ID NO.2: *gag* gene, position 761-822, length 62 nucleotides,

SEQ ID NO.3: *gag* gene, position 1786-1847, length 62 nucleotides,

SEQ ID NO.4: *pol* gene, position 2307-2360, length 54 nucleotides,

SEQ ID NO.5: *pol* gene, position 2376-2434, length 59 nucleotides,

SEQ ID NO.6: *pol* gene, position 2568-2632, length 65 nucleotides,

SEQ ID NO.7: *pol* gene, position 3093-3145, length 53 nucleotides,

SEQ ID NO.8: *pol* gene, position 4131-4207, length 77 nucleotides,

SEQ ID NO.9: *pol* gene, position 4333-4399, length 67 nucleotides,

SEQ ID NO.10: *pol* gene, position 4638-4696, length 59 nucleotides,

SEQ ID NO.11: *pol* gene, position 4884-4984, length 101 nucleotides,

SEQ ID NO.12: *pol* gene, position 5034-5095, length 62 nucleotides,

SEQ ID NO.13: *pol* gene, position 4410-4506, length 97 nucleotides,

The oligonucleotides that are suitable according to the invention preferably have base sequences that are

located within the above-mentioned highly conserved regions or their complementary sequences.

The term "overlapping" should be understood to mean that each of the oligonucleotides according to the invention overlaps at least 10 consecutive bases from one of the highly conserved regions.

The oligonucleotides preferably each overlap one of the regions of SEQ ID NO: 4, 5, 9, 10 and 13, preferably 4, 5, 9 and 10. In another preferred embodiment the oligonucleotides each overlap one of the regions of SEQ ID NO: 6, 8, 10, 11 and 13.

Although the highly conserved regions are stated with reference to a single HIV-1 virus isolate, the term "highly conserved region" of course applies to more than a single specific sequence and thus includes all corresponding regions from various HIV strains or isolates that are related to HIV-1 or HIV-2. Suitable oligonucleotide sequences according to the invention can also have a base sequence which represents a consensus sequence from highly conserved regions of several HI virus isolates or strains. This means that for example a base sequence of several bases corresponds to one HI virus isolate and another base sequence in the same oligonucleotide corresponds to another HI virus isolate. The oligonucleotide then contains heterologous base sequences. The oligonucleotides preferably each contain at least 10 consecutive nucleotides from one of the above-mentioned regions. More preferably they contain 15 to 30 such nucleotides.

The method according to the invention comprises at least

the following steps:

- (a) contacting a sample with the oligonucleotide(s) under such conditions that the oligonucleotide(s) hybridizes/hybridize with the HIV nucleic acids selected from HIV-1 or/and HIV-2 that are present in the sample,
- (b) determining the presence or/and the amount of HIV nucleic acids in the sample.

An amplification step is preferably carried out in step (b).

In a preferred embodiment of the inventive method the subtype-independent or/and species-independent detection of HI virus nucleic acids is made possible by using at least two oligonucleotides according to the invention. These oligonucleotides are preferably used as amplification primers in such a way that they for example each hybridize near to one end of the highly conserved regions and thus generate an amplification product in the amplification (preferably PCR) which corresponds to a section of the respective highly conserved region. This amplification product and thus the HIV-specific nucleic acid can then be detected with the aid of a probe which is either one of the oligonucleotides used as a primer or an additional oligonucleotide which hybridizes within the amplified sequence. The advantage of this preferred embodiment is that a single oligonucleotide or primer pair is sufficient to detect HI viruses independent of subtype or/and species.

Instead of using a probe to detect the amplification product, DNA-binding reagents such as a DNA-binding dye

(e.g. Sybergreen) can also for example be used for the detection (WO97/46707).

According to another preferred embodiment of the method of the invention oligonucleotide combinations or primer pairs are used of which only one oligonucleotide primer is located within one of the conserved regions whereas the other primer is located outside such that the amplification product that is generated in this manner is only partially composed of a base sequence from one of the highly conserved regions. The latter primer is preferably subtype-specific and/or species-specific and thus such combinations can be used to specifically detect subtypes or the species.

In this case preferably two or more primer pairs are used as the oligonucleotide combination of which at least two oligonucleotides each contain at least 10 consecutive nucleotides from a sequence (i), (ii), (iii) or sequences that are complementary thereto as described above. The totality of the primer combinations thus allows a detection of HI viruses that is independent of subtype or/and species.

In a further preferred embodiment a suitable oligonucleotide combination for the method according to the invention comprises two, preferably three oligonucleotides (e.g. a primer pair or a primer pair plus probe) which each contain at least 10 nucleotides from

- (i) the same highly conserved region of the gag gene or of the pol gene of HIV represented by one of the sequences shown in SEQ ID NO:1 to 13,
- (ii) a corresponding region from another HI virus

isolate,

(iii) a corresponding region from a consensus sequence derived from several HI virus isolates, or sequences that are complementary thereto.

The at least one and preferably the at least two oligonucleotide(s) are preferably selected such that they enable a subtype-independent detection of HIV-1 i.e. nucleic acids of at least 7 of the subtypes A, B, C, D, E, F, G, H and O of HIV-1 and preferably all subtypes are detected.

For the subtype-independent detection it is preferable to use at least one and preferably at least two oligonucleotide(s) which each contain at least 10 consecutive nucleotides from

(i) a highly conserved region of the LTR, *gag* gene or *pol* gene of HIV represented by one of the sequences shown in SEQ ID NO: 1, 2, 3, 4, 5, 6, 8, 9, 10, 12 and 13,
(ii) a corresponding region of another HI virus isolate,
(iii) a corresponding region from a consensus sequence derived from several HI virus isolates, or sequences that are complementary thereto.

For the subtype-independent or/and species-independent detection of HIV-1 and HIV-2 it is preferable to use at least one and preferably at least two oligonucleotide(s) which each contain at least 10 consecutive nucleotides from

(i) a highly conserved region of the LTR, *gag* gene or *pol* gene of HIV represented by one of the sequences shown in SEQ ID NO: 1, 2, 3, 4, 5, 7, 9, 10 and 13,
(ii) a corresponding region of another HI virus isolate,

(iii) a corresponding region from a consensus sequence derived from several HI virus isolates, or sequences that are complementary thereto.

It surprisingly turned out that very special oligonucleotides are particularly suitable for the method according to the invention. These oligonucleotides are represented by the sequences shown in SEQ ID NO: 14 to 25. As mentioned above these oligonucleotides can be composed of natural or synthetic nucleic acid building blocks or even of the already mentioned PNA. Preferably at least one of the oligonucleotides used in the method according to the invention carries one or several labels. Suitable labels are fluorescent labels or radioactive labels such as $[^{32}\text{P}]$ -labelled nucleotides.

A further subject matter of the invention is an oligonucleotides which contains at least 10 consecutive nucleotides from

- (i) a highly conserved region of the *pol* gene of HIV represented by one of the sequences shown in SEQ ID NO: 4, 5, 9 or 10,
- (ii) a corresponding region of another HI virus isolate,
- (iii) a corresponding region from a consensus sequence derived from several HI virus isolates, or sequences that are complementary thereto or contain one of the sequences shown in SEQ ID NO: 14 to 25.

The oligonucleotide according to the invention preferably comprises one of the oligonucleotides shown in SEQ ID NO. 14 to 25. The length of the

oligonucleotide according to the invention is preferably 10 to 80 nucleotides. It particularly preferably has about 20 to 30 bases and has a GC content between 40 and 60 %. In addition it is advantageous when the oligonucleotides have no self-complementarity at the 3' end and in addition have no CG run at the 3' end. A GC run is a base sequence which is mainly or exclusively composed of the bases C and G. In addition the oligonucleotides should contain no palindromes. The oligonucleotides according to the invention have preferably no more than 2 mismatches with the corresponding sequences which hybridize with them at the same positions in all subtypes. There are preferably no mismatches at the 3' end of an oligonucleotide according to the invention with nucleotidic acids of the subtypes A, B, C, D, E, F, G, H and O of HIV-1 and/or of subtypes A, B, C and C of HIV-2.

In the case of primer pairs the primers are each selected such that the 5' ends of the primers are positioned no more than 80 bases from one another with respect to the regions in which the primers hybridize on the nucleic acid to be detected. Particularly preferred primer pairs are those in which there is an additional HIV-specific sequence within the region amplified by the primers. This sequence can then be preferably used to detect the amplification products with the aid of a probe which is specific for it. The oligonucleotides according to the invention are preferably provided with at least one of the above-mentioned marker groups.

Thus a further aspect of the present invention is also a combination of two or more oligonucleotides which both have the above-mentioned inventive properties and which in their entirety are suitable for the subtype-

independent and/or species-independent detection of HI viruses.

The invention additionally encompasses combinations of oligonucleotides. Combinations of at least two oligonucleotides are preferred which each contain at least 10 consecutive nucleotides from

- (i) the same highly conserved region of the LTR region, the *gag* gene or *pol* gene of HIV represented by one of the sequences shown in SEQ ID NO: 1 to 13,
- (ii) a corresponding region of another HI virus isolate,
- (iii) a corresponding region from a consensus sequence derived from several HI virus isolates, or sequences that are complementary thereto.

Yet a further aspect of the present invention is a combination of several oligonucleotides where at least two oligonucleotides according to the invention are present and further oligonucleotides which each contain a specific sequence for a single subtype of HIV-1 or/and HIV-2 wherein the entirety of the oligonucleotides in the oligonucleotide combination allows a subtype-independent or/and species-independent detection of HI viruses.

A further object of the present invention is the provision of a reagent kit which contains at least one oligonucleotide according to the invention or an oligonucleotide combination according to the invention as primers or as probes for the detection of HI viruses or their nucleic acids as well as suitable means for carrying out a hybridization and amplification of nucleic acids in a sample.

The invention additionally concerns the use of oligonucleotides or oligonucleotide combinations as primers or/and probes for the detection of HI viruses and especially for the subtype-independent and/or species-independent detection.

It is intended to elucidate the present invention in an illustrative but in no way exhaustive manner by the following examples.

Examples

Example 1

Sensitivity, specificity and dynamic measuring range based on a serial dilution of HIV plasma

In order to examine blood samples, RNA was isolated from HIV-positive plasma with an initial concentration of 15,000 genome equivalents (geq) HIV per ml. This plasma was diluted successively by a factor of 10 in negative plasma and, after sample preparation, each was amplified in duplicate determinations using the corresponding primer pairs. A HIV-negative plasma and water served as controls. In order to determine the specificity a HBV-positive and a HCV-positive plasma was also processed. All samples were measured after amplification (ECL detection, Elecsys®1010).

1. Sample preparation:

Firstly 420 µl plasma was mixed with 80 µl proteinase K (25 mg/ml) and vortexed for several seconds. Then 500 µl lysis buffer (5.4 M guanidinium thiocyanate, 10 mM urea, 10 mM Tris-HCl, 20 % Triton X100, pH 4.4) which contained 1 µg carrier RNA (polyA/ml) was added. The mixture was vortexed and subsequently shaken for 10 minutes at room

temperature. Then 500 μ l isopropanol-MGP was added (6 mg magnetic glass particles in isopropanol). The mixture was again vortexed and subsequently shaken for 20 minutes at room temperature. The MGPs were separated magnetically from the solution. The supernatant was removed and discarded. 750 μ l wash buffer (20 mM NaCl, 20 mM Tris-HCl, pH 7.5, 70 % ethanol) was added, the MGPs were resuspended by vortexing and a magnetic separation was again carried out. The wash process was repeated a total of 5 times and finally 100 μ l DEMC water was added for elution. It was shaken for 15 minutes at 80°C and then an addition magnetic separation was carried out. 10 μ l of the eluate was used for an RT-PCR.

2. Primers and probes which were used:

Primers and probes that were used are shown in the following table 1 which also shows their respective highly conserved region, position in the genome and the amplification product produced with the primer pairs.

Table 1

Primer	highly conserved region	position	amplicon
SK 462* SK 431* SK 102		1359-1388 (30) 1474-1500 (27) 1402-1421 (20)	142 bp
RAR 1032* RAR 1033* RAR 1034*		2961-2992 (32) 3097-3129 (33) 2997-3031 (35)	169 bp
GH A2F** (SEQ ID NO. 14) GH A2R** (SEQ ID NO. 15) GH A2P** (SEQ ID NO. 16)	SEQ ID NO. 8	4143-4162 (20) 4180-4205 (26) 4162-4179 (18)	63 bp
GH A3F** (SEQ ID NO. 17) GH A3R (SEQ ID NO. 18) GH A3P (SEQ ID NO. 19)	SEQ ID NO. 10	4644-4663 (20) 4677-4702 (26) 4663-4677 (15)	59 bp
GH A4F** (SEQ ID NO. 20) GH A4R** (SEQ ID NO. 21) GH A4P** (SEQ ID NO. 22)	SEQ ID NO. 11	4889-4912 (24) 4932-4951 (20) 4913-4931 (19)	63 bp
GH A6F** (SEQ ID NO. 23) GH A6R** (SEQ ID NO. 24) GH A6P (SEQ ID NO. 25)	SEQ ID NO. 13	4412-4437 (26) 4461-4485 (25) 4438-4460 (23)	74 bp

* These primers are all published primers and probes from Roche

** These primers are new primers from the pol region of the HIV genome and correspond to the highly conserved regions shown in table 1.

3. Amplification mix and thermocycler protocol for the RT-PCR:

Master mix:

Reagents	Final concentration in the master mix
5 x bicin buffer	1 x
MnOAc	2.5 mM
dNTPs (incl. dUTP)	200 μ M/600 μ M
forwards primer	0.3 μ M
reverse primer (biotinylated)	0.3 μ M
Tth polymerase	10 units
UNG	2 units
total volume:	100 μ l

Cycler:

10 minutes, 37°C: UNG decontamination
30 minutes, 60°C: reverse transcription
30 seconds, 95°C: denaturation
5 cycles 15 seconds, 95°C: denaturation
 20 seconds, 50°C: hybridization/elongation

30 cycles 15 seconds, 94°C: denaturation
 20 seconds, 60°C: hybridization/elongation
 7 minutes, 72°C: final elongation
 keep at 50°C

4. Detection:

The entire detection reaction was fully automated with the aid of an Elecsys®1010 automated analyser.

Firstly 10 μ l amplificate and 35 μ l denaturation solution (BM-ID-No. 1469053, Boehringer Mannheim) were removed. They were incubated for 5 minutes at 37°C in a reaction vessel and then 120 μ l hybridization solution (BM-ID-No. 1469045, Boehringer Mannheim to which 25 ng/ml ruthenium-labelled probe was added) was added. It was again incubated for 30 minutes at 37°C. Then 35 μ l of an Elecsys® SA magnetic bead solution was added (BM-ID-No. 1719556, Boehringer Mannheim). The solution was incubated for 10 minutes at 37°C. The electrochemiluminescence of 120 μ l of the reaction mixture was measured in the Elecsys® 1010 measuring cell. The appropriate ruthenium-labelled probes were used according to table 1 for hybridization.

5. Results

Result (ECL counts x 100):

Template	SK primer	RAR primer	GH-A2	GH-A3	GH-A4	GH-A6
HIV 15000 copies/ml	5763	294	5786	4209	7981	6809
HIV 1500 copies/ml	626	38	724	466	899	999
HIV 150 copies/ml	184	14	86	164	117	122
HIV 15 copies/ml	58	9	13	27	25	10
HIV 1.5 copies/ml	49	9	14	32	14	10
HIV-negative plasma	70	9	22	38	16	11
HCV-positive plasma	49	9	5	58	16	10
HBV-positive plasma	37	9	5	81	17	10
water	12	9	16	35	15	10

The signals of the new primers have a similar sensitivity to the references. There is a very good signal gradation within the serial dilution.

The increased background with the primer pairs SK and GH-A3 is presumably due to a non-optimal amplification protocol.

Claims

1. Method for the subtype-independent and/or species-independent detection of nucleic acids of HI viruses in a sample by hybridizing the nucleic acids with an oligonucleotide combination comprising two or more oligonucleotides which hybridize specifically with HIV nucleic acids and contain in each case 10 to 80 consecutive nucleotides from
 - (i) the same highly conserved region of the LTR region, of the *gag* gene or of the *pol* gene of HIV represented by one of the sequences shown in SEQ ID NO: 1 to 13,
 - (ii) a corresponding region of another HI virus isolate,
 - (iii) a corresponding region of a consensus sequence derived from several HI virus isolates or sequences which are complementary thereto, and carrying out an enzymatic amplification step.
2. Method as claimed in claim 1,
wherein
it comprises the steps:
 - (a) contacting a sample with the oligonucleotides under such conditions that the oligonucleotides hybridize with the HIV nucleic acids from HIV-1 or/and HIV-2 that are present in the sample,
 - (b) determining the presence and/or the amount of HIV nucleic acids in the sample.

3. Method as claimed in claim 1 or 2,
wherein
only a single oligonucleotide combination is used.
4. Method as claimed in one of the claims 1 to 3,
wherein
the oligonucleotides are selected for a subtype-independent detection in such a manner that at least 7 of the HIV-1 subtypes selected from the subtypes A, B, C, D, E, F, G, H and O and at least 2 of the HIV-2 subtypes selected from the subtypes A, B, C and D are detected.
5. Method as claimed in one of the claims 1 to 3,
wherein
the oligonucleotides are selected for a species-independent detection in such a manner that at least 7 of the HIV-1 subtypes selected from the subtypes A, B, C, D, E, F, G, H and O and additionally at least one of the HIV-2 subtypes selected from the subtypes A, B, C and D are detected.
6. Method for the subtype-independent and/or species-independent detection of nucleic acids of HI viruses in a sample by hybridizing the nucleic acids with two or more oligonucleotide combinations, each oligonucleotide combination comprising a first oligonucleotide which comprises 10 to 80 consecutive nucleotides from
 - (i) a highly conserved region of the LTR region, of the *gag* gene or of the *pol* gene of HIV represented by one of the sequences shown in SEQ ID NO: 1 to 13,

(ii) a corresponding region of another HI virus isolate ,
(iii) a corresponding region of a consensus sequence derived from several HI virus isolates, or sequences which are complementary thereto, and a second oligonucleotide which enables subtype-specific and/or species-specific hybridization with HIV nucleic acids, and carrying out an enzymatic amplification step, wherein the entirety of the oligonucleotide combinations allows a subtype-independent and/or species-independent detection of HI viruses.

7. Method as claimed in claim 6,

wherein

the oligonucleotides are selected for the subtype-independent detection in such a manner that at least 7 of the HIV-1 subtypes selected from the subtypes A, B, C, D, E, F, G, H and O and at least 2 of the HIV-2 subtypes selected from the subtypes A, B, C and D are detected.

8. Method as claimed in claim 7,

wherein

at least two oligonucleotides are used for the detection which contain in each case 10 to 80 consecutive nucleotides from

- (i) a highly conserved region of the LTR gene, of the gag gene or of the pol gene of HIV represented by one of the sequences shown in SEQ ID NO: 2, 4, 5, 6, 8, 9, 10, 12 and 13,
- (ii) a corresponding region of another HI virus isolate ,
- (iii) a corresponding region of a consensus sequence derived from several HI virus isolates

or sequences which are complementary thereto.

9. Method as claimed in claim 6,

wherein

the oligonucleotides are selected for the species-independent detection in such a manner that at least 7 of the HIV-1 subtypes selected from the subtypes A, B, C, D, E, F, G, H and O and additionally at least one of the HIV-2 subtypes selected from the subtypes A, B, C and D are detected.

10. Method as claimed in claim 9,

wherein

at least two oligonucleotides are used for the detection which contain in each case 10 to 80 consecutive nucleotides from

- (i) a highly conserved region of the LTR gene, of the gag gene or of the pol gene of HIV represented by one of the sequences shown in SEQ ID NO: 1, 2, 3, 4, 5, 7, 9, 10 and 13,
- (ii) a corresponding region of another HI virus isolate,
- (iii) a corresponding region of a consensus sequence derived from several HI virus isolates

or sequences which are complementary thereto.

11. Method as claimed in one of the previous claims,

wherein

the oligonucleotides have or contain the sequences shown in SEQ ID NO. 14 to 25.

12. Method as claimed in one of the previous claims,
wherein

at least one oligonucleotide has one or several
labels.

13. Oligonucleotide,

wherein

it comprises 10 to 80 consecutive nucleotides from

- (i) a highly conserved region of the *pol* gene of HIV represented by one of the sequences shown in SEQ ID NO: 4, 5, 9 or 10,
- (ii) a corresponding region of another HI virus isolate,
- (iii) a corresponding region of a consensus sequence derived from several HI virus isolates

or sequences which are complementary thereto,
provided that it does not comprise the nucleotide sequence

CTACTACTCC TTGACTTTGG GGATTG

or its complementary sequence.

14. Oligonucleotide as claimed in claim 13,

wherein

it comprises 10 to 80 consecutive nucleotides from

- (i) a highly conserved region of the *pol* gene of HIV represented by one of the sequences shown in SEQ ID NO: 4, 5 or 9,
- (ii) a corresponding region of another HI virus isolate,
- (iii) a corresponding region of a consensus sequence derived from several HI virus isolates

or sequences which are complementary thereto.

15. Oligonucleotide,

wherein

it comprises at least one of the sequences shown in SEQ ID NO. 14, 16, 17, 18, 20, 22, 23, 24 and 25.

16. Oligonucleotide as claimed in one of the claims 13 to 15,

wherein

it has no mismatches at its 3' end with nucleic acids of the subtypes A, B, C, D, E, F, G, H and O of HIV-1 and of the subtypes A, B, C and D of HIV-2.

17. Oligonucleotide as claimed in one of the claims 13 to 16,

wherein

it has one or several labels.

18. Combination of several oligonucleotides comprising at least two oligonucleotides,

wherein

the at least two oligonucleotides each comprise 10 to 80 consecutive nucleotides from

- (i) a highly conserved region of the LTR region, of the *gag* gene or of the *pol* gene of HIV represented by one of the sequences shown in SEQ ID NO: 1 to 13,
- (ii) a corresponding region of another HI virus isolate,
- (iii) a corresponding region of a consensus sequence derived from several HI virus isolates

or sequences which are complementary thereto and the combination is selected such that it allows an enzymatic amplification.

19. Combination of several oligonucleotides comprising at least two oligonucleotides selected from the oligonucleotides as claimed in one of the claims 13 to 17 and optionally additional oligonucleotides which each contain a sequence that is specific for a single subtype of HIV-1 and/or HIV-2, wherein the entirety of the oligonucleotides allows a subtype-independent and/or species-independent detection of HI viruses.
20. Reagent kit comprising an oligonucleotide as claimed in one of the claims 13 to 17 or an oligonucleotide combination as claimed in claim 18 or 19 as primers and/or probes for the detection of HI viruses or their nucleic acids and suitable means for carrying out a hybridization and amplification of nucleic acids in a sample.
21. Use of oligonucleotides or oligonucleotide combinations as claimed in one of the claims 13 to 19 as primers and/or probes for the subtype-independent and/or species-independent detection of HI viruses.

Abstract

The invention concerns a method for the subtype-independent and/or species-independent detection of HI viruses in a sample using at least one oligonucleotide which contains at least 10 consecutive nucleotides from (i) a highly conserved region of the LTR region, of the *gag* gene or of the *pol* gene of HIV, (ii) a corresponding region of another HI virus isolate, (iii) a corresponding region of a consensus sequence derived from several HI virus isolates or sequences which are complementary thereto.

Express Mail Label No. N/A

Page 1 of 4

Docket No.
18727-US**Declaration and Power of Attorney For Patent Application****English Language Declaration**

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

NEW PRIMERS AND PROBES FOR THE DETECTION OF HIV

the specification of which

(check one)

is attached hereto.
 was filed on April 30, 2001 as United States Application No. or PCT International Application Number 09/830,838
and was amended on October 31, 2001 (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)**Priority Not Claimed**

<u>PCT/EP99/08211</u>	<u>PCT</u>	<u>29 October 1999</u>	<input type="checkbox"/>
<u>(Number)</u> <u>19850186.2</u>	<u>(Country)</u> <u>DE</u>	<u>(Day/Month/Year Filed)</u> <u>30 October 1998</u>	<input type="checkbox"/>
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<u>(Number)</u>	<u>(Country)</u>	<u>(Day/Month/Year Filed)</u>	<input type="checkbox"/>

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

NA

(Application Serial No.)

(Filing Date)

(Application Serial No.)

(Filing Date)

(Application Serial No.)

(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

N/A

(Application Serial No.)

(Filing Date)

(Status)
(patented, pending, abandoned)

(Application Serial No.)

(Filing Date)

(Status)
(patented, pending, abandoned)

(Application Serial No.)

(Filing Date)

(Status)
(patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. (list name and registration number)

George C. Jen, Ph.D., (39,239)
Charles M. Doyle (39,175)

Jennifer A. Haynes, Ph.D., (48,868)

Send Correspondence to: **ROCHE MOLECULAR SYSTEMS, INC.**

Patent Department
1145 Atlantic Avenue
Alameda, California 94501

Direct Telephone Calls to: (name and telephone number)

Charles M. Doyle, Ph.D. (510) 814-2891

Full name of sole or first inventor GERD HABERHAUSEN	Date <i>20/12/02</i>
Sole or first inventor's signature <i>G. Haberhausen</i>	
Residence Jochbergweg 2, D-82393 Iffeldorf, Germany	
Citizenship GERMANY	
Post Office Address Jochbergweg 2, D-82393 Iffeldorf, Germany	

Full name of second inventor, if any PIA KASPER	Date <i>27/12/02</i>
Second inventor's signature <i>P. Kasper</i>	
Residence Sandhofer Strasse 13 a, D-68305 Mannheim, Germany	
Citizenship GERMANY	
Post Office Address Sandhofer Strasse 13 a, D-68305 Mannheim, Germany	

3) Full name of third Inventor, if any
CHRISTOPH KESSLER

Third Inventor's signature

Christoph Kessler

Date

24/12/02

Residence

Schlossbergweg 11, D-82057 Icking, Germany

(D&V)

Citizenship

GERMANY

Post Office Address

Schlossbergweg 11, D-82057 Icking, Germany

Full name of fourth inventor, if any

Fourth Inventor's signature

Date

Residence

Citizenship

Post Office Address

Full name of fifth inventor, if any

Fifth Inventor's signature

Date

Residence

Citizenship

Post Office Address

Full name of sixth inventor, if any

Sixth Inventor's signature

Date

Residence

Citizenship

Post Office Address

SEQUENCE PROTOCOL

(1) GENERAL INFORMATION:

(i) APPLICANT:

- (A) NAME: Boehringer Mannheim GmbH
- (B) ROAD: Sandhofer Strasse 112-132
- (C) CITY: Mannheim-Waldhof
- (E) Country: DE
- (F) ZIP CODE: 68305

(ii) TITLE OF INVENTION: New primers and probes for the detection of HIV

(iii) NUMBER OF SEQUENCES: 25

(iv) COMPUTER-READABLE FORM:

- (A) DATA CARRIER: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 62 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: linear

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 62 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: linear

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 62 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: linear

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- (A) LENGTH: 54 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: linear

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(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 59 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

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- (A) LENGTH: 65 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: linear

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 53 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: linear

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 77 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: linear

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- (A) LENGTH: 67 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: linear

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- (A) LENGTH: 59 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: linear

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- (A) LENGTH: 101 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 62 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

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(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 97 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

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(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

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(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

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(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

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(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

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(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: single strand
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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 base pairs
- (B) TYPE: nucleotide
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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

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(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleotide
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- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleotide
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(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

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09/830838
531 Rec'd PCT/ 30 APR 2001

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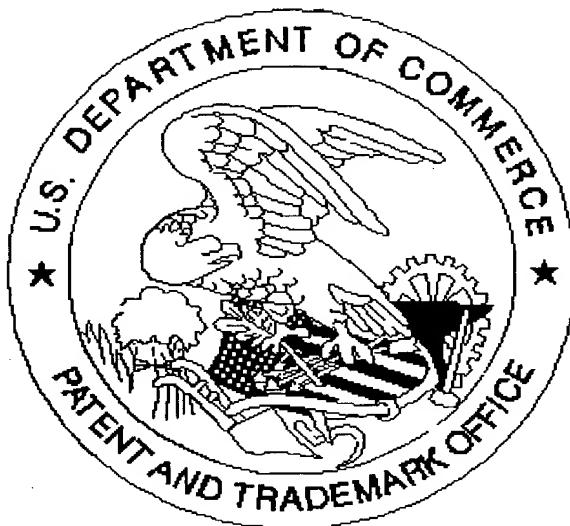
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